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HOT N COLD formulation of biologics for the optimization of drying technologies

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PURPOSE

Biologics are expected to expand their pharmaceutical pipeline, as they offer targeted treatment options for a wide range of diseases [1,2]. However, maintaining the biomolecules' stability throughout process development is a known challenge [3,4]. Thus, providing meaningful understanding of these complex biomolecules through fast and reliable data becomes increasingly crucial, as it paves the way for the success of every biotherapeutic [3,4].

OBJECTIVES

This work aims to assess the impact of different excipients on protein stability during freeze drying (FD) and spray drying (SD) processes, using lysozyme as model drug.

METHODS

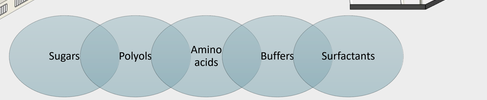
Differential Scanning Fluorimetry (DSF)

Melting temperature (T_m)

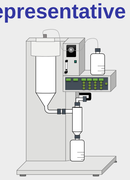
Dynamic Light Scattering (DLS)

Aggregation temperature (T_{agg})*

*for surfactants due to incompatibility with DSF dye



Representative industrial processing stresses



Spray Drying

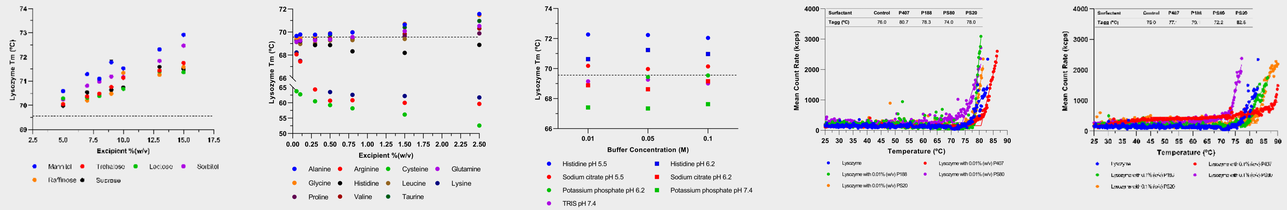
Freeze Drying

Analytical characterization

- Karl Fisher
- Scanning electron microscopy (SEM)
- Size exclusion chromatography (SEC)
- Lysozyme Activity Kit

RESULTS

Effect of different classes of excipients on lysozyme's T_m and T_{agg}



(a) (b) (c) (d) (e)

Fig. 1 – Melting temperatures of lysozyme in PBS buffer for different (a) sugars and polyols, (b) amino acids, and (c) buffers obtained with DSF and thermal ramps of lysozyme for different surfactants at (d) 0.01% (w/v) and (e) 0.1% (w/v) obtained with DLS.

- ❑ All sugars and polyols stabilized lysozyme (↑T_m) but some amino acids showed a destabilizing effect (↓ T_m).
- ❑ Buffer type and pH significantly affected lysozyme's T_m. However, within the tested range, the buffer concentration did not have a significant impact.
- ❑ All surfactants tested ↑ lysozyme's T_{agg}, except for polysorbate 80 (PS80), possible due to its thermal oxidation throughout the thermal ramp.

Effect of different classes of excipients on Spray Drying and Freeze Drying

Freeze drying → Large, irregular shaped particles

Spray drying → Small spherical particles with **controlled size and morphology**

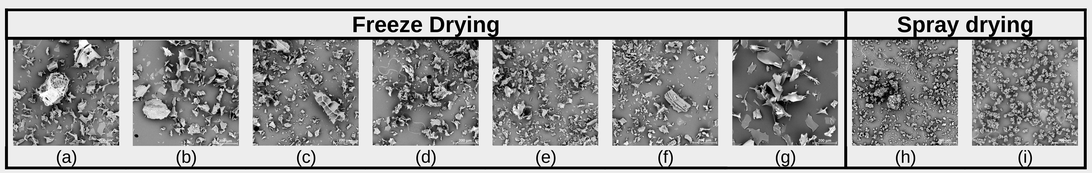
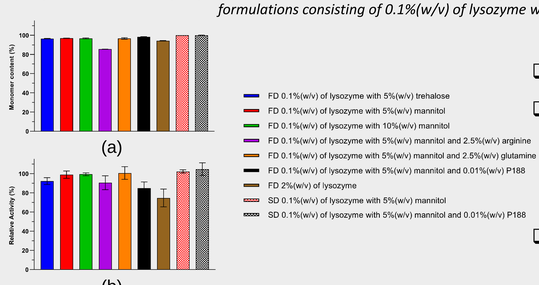


Fig. 2 – SEM images of freeze dried lysozyme formulations in in 0.05 M Histidine pH 5.5 consisting of 0.1%(w/v) of lysozyme with (a) 5%(w/v) trehalose, (b) 5%(w/v) mannitol, (c) 10%(w/v) mannitol, (d) 5%(w/v) mannitol and 2.5%(w/v) arginine, (e) 5%(w/v) mannitol and 2.5%(w/v) glutamine, and (f) 5%(w/v) mannitol and 0.01%(w/v) poloxamer 188 and (g) 2%(w/v) of lysozyme as well as spray dried formulations consisting of 0.1%(w/v) of lysozyme with (h) 5% mannitol and (i) 5%(w/v) mannitol and 0.01%(w/v) poloxamer 188.



(a)

(b)

Fig. 3 – (a) Lysozyme monomer content and (b) relative activity for the different freeze dried and spray dried formulations in 0.05 M Histidine pH 5.5.

- ❑ FD resulted in higher water content (1.3 - 4.7%) when compared to SD (0.8%).
- ❑ SEC data was aligned with T_m and T_{agg} data → All FD trials resulted in a small decrease in monomer content, but the combination of arginine with mannitol as well as the formulation without excipients had a more significant impact on lysozyme's stability.
- ❑ Enzymatic activity results after FD were consistent with SEC data, except for the trial with P188. P188 negatively impacted enzymatic activity.
- ❑ SD had a negligible effect on lysozyme's stability and enzymatic activity.

CONCLUSIONS

This work allowed to understand the interplay of a wide range of excipients in maintaining protein activity and stability during processing, forecasting scalable drug product development.

- ❑ Both T_m and T_{agg} proved to be suitable parameters to predict the impact of different excipients on proteins as its variation was in general aligned with the excipients' effect on the drying processes.
- ❑ P188 showed compatibility issues with FD, which impacted protein bioactivity and was not observed during SD. This effect was possibly related to its local concentration caused by the slow freezing rates.
- ❑ Unlike FD, SD allowed to control particle characteristics and for the same formulation, the later induced less protein degradation, which validates it as a mild process for biologics.

This study validated the importance of considering the specific interactions between excipients and proteins, as well as the compatibility of formulations with the selected processing method to maintain proteins' stability and function

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